

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

STRAUS, Alexander
Becker.Kurig.Straus
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ALLEMAGNE

Date of mailing (day/month/year) 12 March 2001 (12.03.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference NO 6436/WO	
International application No. PCT/EP00/01744	
	International filing date (day/month/year) 01 March 2000 (01.03.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☒ the name ☒ the address ☐ the nationality ☐ the residence

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3. Further observations, if necessary:

New agent has been appointed.

4. A copy of this notification has been sent to:

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<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input checked="" type="checkbox"/> other: former agent

The International Bureau of WIPO
34, chemin des Colombettes
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Authorized officer

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003890524

PATENT COOPERATION TREATY

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 October 2000 (23.10.00)	
International application No. PCT/EP00/01744	Applicant's or agent's file reference NO 6436/WO
International filing date (day/month/year) 01 March 2000 (01.03.00)	Priority date (day/month/year) 12 March 1999 (12.03.99)
Applicant GARCIA-RODENAS, Clara, L. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
06 October 2000 (06.10.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

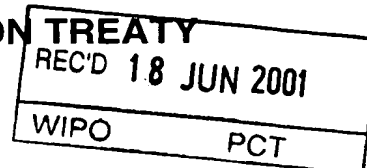
made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer R. E. Stoffel</p> <p>Telephone No.: (41-22) 338.83.38</p>
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09/936446

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference NO 6436/WO		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP00/01744	International filing date (day/month/year) 01/03/2000	Priority date (day/month/year) 12/03/1999	
International Patent Classification (IPC) or national classification and IPC A23J3/34			
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the report
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 06/10/2000	Date of completion of this report 15.06.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer De Jong, E Telephone No. +31 70 340 3849 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01744

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-10 as originally filed

Claims, No.:

1-7 as received on 05/03/2001 with letter of 05/03/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP00/01744

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-7
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations
see separate sheet

Ad I

The subject-matter of the claims does not meet the requirements of Rule 70.2(c) PCT, because there is no support in the original disclosure for the phrase "degree of hydrolysis in a range of from about 10% to less than 50% by weight" (underlining added) in claim 1.

Ad V

Reference is made to the following documents:

D1 = US-A-4 977 137

D2 = US-A-5 514 655

D3 = CA-A-2 163 379

D4 = Hamosh M., Journal of Nutrition 1997, p.971S-974S

D5 = Schanbacher F.L. et al., International Dairy Journal 1998, p.393-403

D6 = EP-A-0 852 913

The subject-matter of the claims is considered to be anticipated and not to involve an inventive step (Articles 33(2) and (3) PCT) in view of D1-D6:

D1 discloses (see claims 1-27) the use of lactoferrin as a dietary ingredient to a formula, e.g. hydrolysed casein formula (see col.11 l.54-col.12 l.23), to promote growth of the gastrointestinal tract of human infants and newborn nonhuman animals immediately on birth.

D2 discloses (see claims 1-2, col.19 l.1-67) a nutritional enteral product which contains a protein system comprising a soy protein hydrolysate and intact protein (sodium caseinate, pea protein, whey protein concentrate). The product provides enteral nutritional support for cancer patients.

D3 discloses a nutritional composition for oral or enteral administration, comprising a source of dietary nitrogen providing 15-25% of the total energy, carbohydrates providing 60-75% of the total energy and lipids providing 10-20%. The dietary nitrogen component comprises 20-30% by weight free amino acids, 60-75% hydrolysed casein and 5-15% intact caseinate protein. The composition provides improved digestion and adsorption.

D4-D6 refer to the importance of bioactive peptides for infant formulas and functional foods (e.g. casein rich in TGF-[SPEC0803]2 for treatment of Crohn's disease).

Applicant: Société Des Produits Nestlé S.A.
Our file: 80300 WO
PCT/EP00/01744

Claims

1. A nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains
 - a mixture of dietary protein hydrolysates having a degree of hydrolysis in a range of from about 10 % to less than 50 % by weight and being in form of a mixture of different size peptides and free amino acids, the free amino acids being present in an amount of up to about 20 % (each calculated as nitrogen x 6,25),
 - intact proteins being partly in form of bioactive peptides.
2. The composition of claim 1, wherein the dietary protein hydrolysates contain at least about 5% (by weight, of the total protein content calculated as nitrogen x 6,25) of hydrolysate having a degree of hydrolysis of about 40 % and at least about 5 % of hydrolysates having a lesser degree of hydrolysis.
3. The composition according to any of the preceding claims, wherein the intact proteins are present in an amount of at least about 5 % by weight of the total protein content.
4. The composition according to any of the preceding claims, wherein the intact proteins are milk proteins, whey proteins, caseins and bioactive proteins, such as TGF- β .
5. The composition according to any of the preceding claims, wherein bioactive peptides represent at least about 0.1 to about 4 ng/mg total protein.
6. The composition according to any of the preceding claims, which contains a source of protein providing 5 to 30 % of the total energy, a source of carbohydrates, which provides 40 to 80 % of the total energy, a source of lipids, which provides 5 to 55 % of the total energy, minerals and vitamins to meet daily requirements.

7. Use of a composition according to any of the preceding claims for the preparation of a nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A23J 3/34, A23L 1/305	A1	(11) International Publication Number: WO 00/54603 (43) International Publication Date: 21 September 2000 (21.09.00)
(21) International Application Number: PCT/EP00/01744 (22) International Filing Date: 1 March 2000 (01.03.00) (30) Priority Data: 99200753.4 12 March 1999 (12.03.99) EP (71) Applicant (for all designated States except US): SOCIETE DES PRODUITS NESTLE S.A. [CH/CH]; P.O. Box 353, CH-1800 Vevey (CH). (72) Inventors; and (75) Inventors/Applicants (for US only): GARCIA-RODENAS, Clara, L. [ES/CH]; Résidence Le Frêne A, CH-1606 Forel (CH). FINOT, Paul-André [FR/CH]; Rte Tirage 1, CH-1806 St-Légier-La Chiésaz (CH). MAIRE, Jean-Claude [CH/CH]; 1, ch. de Rueyres, CH-1092 Belmont s/Lausanne (CH). BALLEVRE, Olivier [FR/CH]; Rte de Cojonnex 16B, CH-1000 Lausanne 25 (CH). DONNET-HUGHES, Anne [GB/CH]; Rue Châtel-St-Denis 29B, CH-1806 Saint-Légier (CH). HASCHKE, Ferdinand [AT/CH]; Rte de Lavaux 254, CH-1095 Lutry (CH). (74) Agent: LOCK, Graham; 55, Avenue Nestlé, CH-1800 Vevey (CH).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: NUTRITIONAL COMPOSITION INTENDED FOR SPECIFIC GASTRO-INTESTINAL MATURATION IN PREMATURE MAMMALS		
(57) Abstract A nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.		

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Nutritional composition intended for specific gastro-intestinal maturation in premature mammals

Field of the Invention

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This invention relates to an enteral composition containing peptides in an adapted profile size, bioactive peptides, intact proteins, and free amino acids intended for specific gastro-intestinal maturation in premature mammals.

Background to the Invention

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Nutritional compositions based upon hydrolysates of proteins such as milk or soy, are commonly used in infant and clinical nutrition and particularly in hypoallergenic formulas and formulas for patients suffering from various intestinal absorption problems. It is also known to use free amino acids in nutritional compositions for example for patients suffering from particular diseases or conditions such as inflammatory bowel disease, intractable diarrhoea, short bowel syndrome, and the like. Accordingly, amino acids are used either alone or in combination with protein or protein hydrolysates. Protein hydrolysates or free amino acid mixtures are also mainly used in particular cases such as allergy to whole proteins.

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Another interest in using protein hydrolysates in nutrition is due to the fact that they are more rapidly absorbed in the intestine than whole protein or free amino acids. However, it is not clear whether this faster absorption translates into better nitrogen utilisation since studies carried out to date have provided conflicting results (Collin-Vidal *et al*; 1994; Endocrinol. Metab., 30, E 907-914). Further, this interest is in the sense of providing a source of amino acids to meet the general amino acids needs of the patient and not to specifically provide for the needs of individual gastro-intestinal maturation.

Summary of the Invention

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Accordingly, on one aspect, this invention provides a nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a

mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.

5 In this composition, the dietary protein hydrolysates are preferably in the form of a mixture of different size peptides, free amino acids or a mixture thereof. The dietary protein hydrolysates may be hydrolysates of animal proteins (such as milk proteins, meat proteins and egg proteins), or vegetable proteins (such as soy proteins, wheat proteins, rice proteins, and pea proteins). The preferred source is milk protein. The dietary protein hydrolysates can be used as
10 such or like peptide fractions isolated from them.

The hydrolysed proteins may comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of hydrolysates having a lesser degree of
15 hydrolysis. Free amino acids are preferably in an amount of about 0 to 20 % by weight of the total protein content (N x 6.25).

The intact proteins may be individual or enriched animal or vegetable protein fractions comprising whole milk, caseins, whey proteins, soy proteins or
20 rice proteins, for example. They are preferably in an amount of at least about 5 % of the total protein content (N x 6.25).

The intact protein fraction may contain bioactive peptides such as TGF- β 2 or a source of bioactive peptides such as beta-casein liberated in the gut by
25 enzymatic hydrolysis. The final TGF- β 2 concentration may be in the range of 0.1 to 4 ng/mg total protein, preferably about 1 to 2.5 ng/mg.

The nutritional composition may also contain a source of fat and a source of carbohydrates. This composition preferably contains a source of protein
30 providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.

35 In another aspect, this invention provides the use of a selected mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides for the preparation of a nutritional enteral composition for

favoring the growth and maturation of non- or pre-mature gastro-intestinal tracts of young mammals.

5 The nutritional composition also intends to cover very high nutrient needs for growth and development during that stage. It ensures optimal digestion and utilization (for tissue accretion) of the protein source and intends to minimize the nitrogen waste of the organism. Moreover, a mixture of intact protein, protein hydrolysates, bioactive peptides and free amino acids provides a better source of amino acids to meet the general amino acid needs of the patient in addition to
10 specifically favor the maturation of individual organs.

Embodiments of the invention are now described by way of example only.

Detailed Description of the Invention

15 In the specification, the term "degree of hydrolysis" (DH) means the percentage of nitrogen in the form of free alpha-amino nitrogen as compared to total nitrogen. It is a measure of the extent to which the protein has been hydrolysed.

20 The term bioactive peptide relates to i) a protein or peptide present as such in the preparation and demonstrating specific functional properties or ii) a protein or peptide containing an amino acid sequence with specific properties, this sequence being liberated in the gastro-intestinal tract during the natural process of
25 digestion.

According to a first aspect of the invention, the nutritional composition comprises as a source of protein a selected mixture of intact protein being partly in the form of bioactive peptides and dietary protein hydrolysates having a degree
30 of hydrolysis in the range of about 5% to about 50% and free amino acids. The non-protein nitrogen concentration of the protein source can be comprised between 10% and 95% of the total nitrogen. Such protein source maximizes the area of the intestine in which the protein is digested and optimizes protein synthesis in the gut and peripheral tissues.

The nutritional composition can also contain a carbohydrate source, a fat source, vitamins and minerals.

The intact protein may be any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); or combinations thereof. Milk proteins such as casein and whey protein are particularly preferred. They are preferably in an amount at least of about 5 % of the total protein content (calculated as Nitrogen x 6.25).

Dietary protein in the form of intact protein is found to increase the rate of muscle protein synthesis as compared to protein hydrolysates.

The dietary protein hydrolysates may come from any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); or combinations thereof. Milk proteins such as casein and whey protein are particularly preferred. The hydrolysed dietary proteins may comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of hydrolysates having a lesser degree of hydrolysis.

In particular, hydrolysates having a degree of hydrolysis of about 10% to about 15%, are found to increase relative weight of the liver as compared to free amino acid mixes. Hydrolysates having a degree of hydrolysis of about 15% to about 25% are found to increase the concentration of protein in the jejunum, the relative weight of the jejunum and the rate of protein synthesis in the jejunum. Highly hydrolysed protein which has a degree of hydrolysis of greater than 25% or which contains more than 25% by weight of di- and tri-peptides, more preferably greater than 30%, is found to increase the rate of protein synthesis in the jejunum and the duodenum; particularly the duodenum.

The dietary protein hydrolysates may be produced using procedures which are well known in the art or may be obtained commercially. For example, nutritional formulas containing hydrolysates having a degree of hydrolysis less than about 15% are commercially available from Nestlé Nutrition Company under the trade mark Peptamen®. Hydrolysates having a degree of hydrolysis above about 15% may be prepared using the procedure described in EP 0322589.

The dietary protein hydrolysate source may also be in the form of a mix of free amino acids; preferably such that the mix provides a balanced amino acid profile. Free amino acids are preferably in an amount of about 0 to 20 % by weight of the total protein content (calculated as Nitrogen x 6.25).

Dietary protein in the form of a mix of free amino acids is found to increase the relative weight of the jejunum and the rate of protein synthesis in the jejunum.

The source of total proteins preferably provides about 5% to about 30% of the energy of the nutritional composition; for example about 10% to about 20% of the energy. The remaining energy of the nutritional composition may be provided in the form of carbohydrates and fats.

If the nutritional composition includes a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional composition; for example about 20% to about 50% of the energy. The lipids making up the fat source may be any suitable fat or fat mixture. Vegetable fats are particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins, and the like. Animal fats such as milk fats may also be added if desired. The lipids may also include medium-chain triglycerides; for example up to about 60 % by weight of lipids as medium-chain triglycerides. Fractionated coconut oil is a suitable source of medium-chain triglycerides.

A source of carbohydrate may be added to the nutritional composition. It preferably provides about 40% to about 80% of the energy of the nutritional composition. Any suitable carbohydrates may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, and maltodextrins, and mixtures thereof.

Dietary fibre may also be added if desired. If used, it preferably comprises up to about 5% of the energy of the nutritional composition. The dietary fibre may be from any suitable origin, including for example soy, pea, oat, pectin, guar gum, and gum arabic.

Suitable vitamins and minerals may be included in the nutritional composition in an amount to meet the appropriate guidelines.

One or more food grade emulsifiers may be incorporated into the nutritional composition if desired; for example diacetyl tartaric acid esters of mono-diglycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included.

The nutritional composition is preferably enterally administrable; for example in the form of a powder, a liquid concentrate, a ready-to-drink, or a ready-to-administer beverage.

The nutritional composition may be prepared in any suitable manner. For example, it may be prepared by blending together the source of dietary protein, the carbohydrate source, and the fat source in appropriate proportions. If used, the emulsifiers may be included in the blend. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any lipophilic vitamins, emulsifiers and the like may be dissolved into the fat source prior to blending. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The temperature of the water is conveniently about 50°C to about 80°C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. The liquid mixture is then homogenised; for example in two stages.

The liquid mixture may then be thermally treated to reduce bacterial loads, by rapidly heating the liquid mixture to a temperature in the range of about 80°C to about 150°C for about 5 seconds to about 5 minutes, for example. This may be carried out by steam injection, autoclave or by heat exchanger; for example a plate heat exchanger.

Then, the liquid mixture may be cooled to about 60°C to about 85°C; for example by flash cooling. The liquid mixture may then be again homogenised; for example in two stages at about 7 MPa to about 40 MPa in the first stage and about 2 MPa to about 14 MPa in the second stage. The homogenised mixture may then be further cooled to add any heat sensitive components; such as vitamins and minerals. The pH and solids content of the homogenised mixture is conveniently standardised at this point.

If it is desired to produce a powdered nutritional composition, the homogenised mixture is transferred to a suitable drying apparatus such as a spray

drier or freeze drier and converted to powder. The powder should have a moisture content of less than about 5% by weight.

5 If it is desired to produce a liquid composition, the homogenised mixture is preferably aseptically filled into suitable containers by pre-heating the homogenised mixture (for example to about 75 to 85°C) and then injecting steam into the homogenised mixture to raise the temperature to about 140 to 160°C; for example at about 150°C. The homogenised mixture may then be cooled, for example by flash cooling, to a temperature of about 75 to 85°C. The
10 homogenised mixture may then be homogenised, further cooled to about room temperature and filled into containers. Suitable apparatus for carrying out aseptic filling of this nature is commercially available. The liquid composition may be in the form of a ready to feed composition having a solids content of about 10 to about 14% by weight or may be in the form of a concentrate; usually of solids
15 content of about 20 to about 26% by weight. Flavours may be added to the liquid compositions so that the compositions are provided in the form of convenient, flavoursome, ready-to-drink beverages.

20 In another aspect, this invention provides a method for increasing protein concentration and synthesis in the small intestine, the method comprising administering to a pre-mature or non-mature mammal an effective amount of a nutritional composition containing a dietary protein hydrolysates having a degree of hydrolysis of less than 50 % and intact proteins being partly in the form of bioactive peptides. Further, the dietary protein hydrolysate preferably has a non-
25 protein nitrogen concentration of at least about 85% of total nitrogen. Non protein nitrogen is defined as the nitrogen fraction not recovered as a precipitate after acidification.

30 Preferably, the method may be used to treat premature or non-mature young mammals to promote growth and maturation of the gastro-intestinal tract. Additionally, the method can also apply to situations encountered in clinical nutrition when alterations of the normal growth or turnover of the gut mucosa occur, e.g. after long term total parenteral nutrition or malnutrition.

35 The nutritional enteral composition also intends to cover very high nutrient needs for growth, development and maintenance during those situations. It

ensures optimal digestion and utilization (for tissue accretion) of the protein source and intends to minimize the nitrogen waste of the organism. The composition may also be used for patients with gut mucosa damage.

5 The amount of the nutritional composition to be administered will vary depending upon the state of maturation or growth of the gut of the mammal.

Example 1

Whole protein

10 An amount of 5 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in demineralised water at 55°C to obtain protein concentration ($N \times 6.38$) of 10% by weight. The pH of the dispersion is adjusted by the addition of 190 g of calcium hydroxide and the dispersion is cooled to room temperature. The proteins are then dried by lyophilisation and
15 packaged into metal cans.

The whole proteins have a degree of hydrolysis of about 4.41% and a non protein nitrogen concentration of about 1.1% on the basis of total nitrogen.

Hydrolysate 1

20 An amount of 6.25 kg of whey protein (obtained from Meggle GmbH) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 8.2 by the addition of 1.8 litres of 2M $\text{Ca}(\text{OH})_2$. The proteins are then hydrolysed using 30 g of trypsin (Salt free pancreatic trypsin which has an activity of 6.8 AU/g and a chymotrypsin content of less than 5% and which is
25 obtainable from Novo Nordisk Ferment AG, Dittigen, Switzerland). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to
30 16°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans. The hydrolysate has a degree of hydrolysis of about 14% and a non protein nitrogen concentration of about 54.5% on the basis of total nitrogen.

Hydrolysate 2

35 An amount of 6.25 kg of whey protein (obtained from Meggle GmbH) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is

adjusted to 7.5 by the addition of 1.6 litres of 1M Ca(OH)₂ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 50 g of trypsin (obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated and non-hydrolysed protein is denatured, by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes.

The mixture is then cooled to 56°C and hydrolysed again for 1 hour using 50g of trypsin at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to 18°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

The hydrolysate has a degree of hydrolysis of about 17.3% and a non protein nitrogen concentration of about 65.9% on the basis of total nitrogen.

Hydrolysate 3

An amount of 6.25 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 7.5 by the addition of 1.6 litres of 1M Ca(OH)₂ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 250 g of Alcalase 2.4L (EC 940459 - obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. For the first hour of the reaction, the pH is regulated to 7.6 by the addition of 1.6N NaOH and 0.4N KOH.

An amount of 250g of Neutrase 0.5L (obtainable from Novo Nordisk Ferment AG) is added and the proteins are further hydrolysed for 4 hours at 50°C. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes. The reaction mixture is then cooled to 55°C.

The pH of the reaction mixture is adjusted to 7.33 by the addition of 1.6N NaOH and 0.4N KOH and the reaction mixture hydrolysed again for 4 hours using 100g of pancreatin at 55°C. During the reaction, the pH is regulated to 7.5 by the addition of 1M NaOH. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5

minutes. The mixture is then cooled to 4°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

5 The hydrolysate has a degree of hydrolysis of about 35% and a non protein nitrogen concentration of about 92.6% on the basis of total nitrogen.

Example 2

10 In order to obtain a nutritional composition intended for specific gastro intestinal maturation in premature mammals, the following mixture is prepared :

i) 14.5 g/ 100 g powder total protein content:

10 % hydrolysate 2 as prepared in example 1,

40 % hydrolysate 3 as prepared in example 1,

15 50 % intact proteins (containing 1 ppm TGFβ2),

ii) 26 g/ 100 g powder of fat:

40 % medium chain triglycerides

20 60 % long chain triglycerides

iii) 53.6 g/ 100 g powder carbohydrates

65 % lactose

35 % maltodextrins

25 iv) and vitamins, minerals to meet daily requirements.

Claims

1. A nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.
2. A composition according to claim 1, wherein the dietary protein hydrolysates are in the form of a mixture of different size peptides, free amino acids or a mixture thereof.
3. A composition according to claim 2, wherein the dietary protein hydrolysates contain at least about 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least about 5 % of hydrolysates having a lesser degree of hydrolysis.
4. A composition according to claims 2 or 3, wherein free amino acids are in an amount of about 0 to about 20 % by weight of the total protein content (calculated as Nitrogen x 6.25).
5. A composition according to any of claims 1 to 4, wherein the intact proteins are in an amount of at least about 5% by weight of the total protein content (calculated as Nx6.25).
6. A composition according to any of claims 1 to 5, wherein the intact proteins are milk proteins, whey proteins, caseins and bioactive peptides such as TGF- β 2.
7. A composition according to any of claims 1 to 6, wherein bioactive peptides represent at least about 0.1 to about 4 ng/mg total protein.
8. A composition according to any of claims 1 to 7 which contains a source of protein providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.

- 5 9. Use of a selected mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides as protein source in the preparation of a nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals.
- 10 10. Use according to claim 9, wherein the dietary protein hydrolysates are in the form of a mixture of different size peptides, free amino acids or a mixture thereof.
- 15 11. Use according to claim 9 or 10, wherein the dietary protein hydrolysates comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of hydrolysates having a lesser degree of hydrolysis.
- 20 12. Use according to any of claim 9 to 11, wherein free amino acids are in an amount of about 0 to about 20 % by weight of the total protein content (N x 6.25)
13. Use according to any of claims 9 to 12, wherein the intact proteins are in an amount of at least about 5% of the total protein content.
- 25 14. Use according to any of claims 9 to 13, wherein the the intact proteins are milk proteins, whey proteins, caseins and bioactive peptides such as TGF- β 2.
15. Use according to any of claims 9 to 14, wherein bioactive peptides represent about 0.1 to about 4 ng/mg total protein.
- 30 16. Use according to any of claims 9 to 15, in which the nutritional composition contains a source of protein providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.
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(54) Title: **NUTRITIONAL COMPOSITION INTENDED FOR SPECIFIC GASTRO-INTESTINAL MATURATION IN PREMA-TURE MAMMALS**

(57) Abstract: A nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.

Nutritional composition intended for specific gastro-intestinal maturation in premature mammals

Field of the Invention

5 This invention relates to an enteral composition containing peptides in an adapted profile size, bioactive peptides, intact proteins, and free amino acids intended for specific gastro-intestinal maturation in premature mammals.

Background to the Invention

10 Nutritional compositions based upon hydrolysates of proteins such as milk or soy, are commonly used in infant and clinical nutrition and particularly in hypoallergenic formulas and formulas for patients suffering from various
15 intestinal absorption problems. It is also known to use free amino acids in nutritional compositions for example for patients suffering from particular diseases or conditions such as inflammatory bowel disease, intractable diarrhoea, short bowel syndrome, and the like. Accordingly, amino acids are used either alone or in combination with protein or protein hydrolysates. Protein hydrolysates
20 or free amino acid mixtures are also mainly used in particular cases such as allergy to whole proteins.

25 Another interest in using protein hydrolysates in nutrition is due to the fact that they are more rapidly absorbed in the intestine than whole protein or free amino acids. However, it is not clear whether this faster absorption translates into better nitrogen utilisation since studies carried out to date have provided conflicting results (Collin-Vidal *et al*; 1994; Endocrinol. Metab., 30, E 907-914). Further, this interest is in the sense of providing a source of amino acids to meet the general amino acids needs of the patient and not to specifically provide for
30 the needs of individual gastro-intestinal maturation.

Summary of the Invention

35 Accordingly, on one aspect, this invention provides a nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a

mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.

5 In this composition, the dietary protein hydrolysates are preferably in the form of a mixture of different size peptides, free amino acids or a mixture thereof. The dietary protein hydrolysates may be hydrolysates of animal proteins (such as milk proteins, meat proteins and egg proteins), or vegetable proteins (such as soy proteins, wheat proteins, rice proteins, and pea proteins). The preferred source is milk protein. The dietary protein hydrolysates can be used as
10 such or like peptide fractions isolated from them.

The hydrolysed proteins may comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of hydrolysates having a lesser degree of
15 hydrolysis. Free amino acids are preferably in an amount of about 0 to 20 % by weight of the total protein content (N x 6.25).

The intact proteins may be individual or enriched animal or vegetable protein fractions comprising whole milk, caseins, whey proteins, soy proteins or
20 rice proteins, for example. They are preferably in an amount of at least about 5 % of the total protein content (N x 6.25).

The intact protein fraction may contain bioactive peptides such as TGF- β 2 or a source of bioactive peptides such as beta-casein liberated in the gut by
25 enzymatic hydrolysis. The final TGF- β 2 concentration may be in the range of 0.1 to 4 ng/mg total protein, preferably about 1 to 2.5 ng/mg.

The nutritional composition may also contain a source of fat and a source of carbohydrates. This composition preferably contains a source of protein
30 providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.

In another aspect, this invention provides the use of a selected mixture of
35 dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides for the preparation of a nutritional enteral composition for

favoring the growth and maturation of non- or pre-mature gastro-intestinal tracts of young mammals.

The nutritional composition also intends to cover very high nutrient needs for growth and development during that stage. It ensures optimal digestion and utilization (for tissue accretion) of the protein source and intends to minimize the nitrogen waste of the organism. Moreover, a mixture of intact protein, protein hydrolysates, bioactive peptides and free amino acids provides a better source of amino acids to meet the general amino acid needs of the patient in addition to specifically favor the maturation of individual organs.

Embodiments of the invention are now described by way of example only.

Detailed Description of the Invention

In the specification, the term "degree of hydrolysis" (DH) means the percentage of nitrogen in the form of free alpha-amino nitrogen as compared to total nitrogen. It is a measure of the extent to which the protein has been hydrolysed.

The term bioactive peptide relates to i) a protein or peptide present as such in the preparation and demonstrating specific functional properties or ii) a protein or peptide containing an amino acid sequence with specific properties, this sequence being liberated in the gastro-intestinal tract during the natural process of digestion.

According to a first aspect of the invention, the nutritional composition comprises as a source of protein a selected mixture of intact protein being partly in the form of bioactive peptides and dietary protein hydrolysates having a degree of hydrolysis in the range of about 5% to about 50% and free amino acids. The non-protein nitrogen concentration of the protein source can be comprised between 10% and 95% of the total nitrogen. Such protein source maximizes the area of the intestine in which the protein is digested and optimizes protein synthesis in the gut and peripheral tissues.

The nutritional composition can also contain a carbohydrate source, a fat source, vitamins and minerals.

5 The intact protein may be any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); or combinations thereof. Milk proteins such as casein and whey protein are particularly preferred. They are preferably in an amount at least of about 5 % of the total protein content (calculated as Nitrogen x 6.25).
10 Dietary protein in the form of intact protein is found to increase the rate of muscle protein synthesis as compared to protein hydrolysates.

The dietary protein hydrolysates may come from any suitable dietary protein; for example animal proteins (such as milk proteins, meat proteins and
15 egg proteins); vegetable proteins (such as soy protein, wheat protein, rice protein, and pea protein); or combinations thereof. Milk proteins such as casein and whey protein are particularly preferred. The hydrolysed dietary proteins may comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of
20 hydrolysates having a lesser degree of hydrolysis. In particular, hydrolysates having a degree of hydrolysis of about 10% to about 15%, are found to increase relative weight of the liver as compared to free amino acid mixes. Hydrolysates having a degree of hydrolysis of about 15% to about 25% are found to increase the concentration of protein in the jejunum, the relative
25 weight of the jejunum and the rate of protein synthesis in the jejunum. Highly hydrolysed protein which has a degree of hydrolysis of greater than 25% or which contains more than 25% by weight of di- and tri-peptides, more preferably greater than 30%, is found to increase the rate of protein synthesis in the jejunum and the duodenum; particularly the duodenum.

30 The dietary protein hydrolysates may be produced using procedures which are well known in the art or may be obtained commercially. For example, nutritional formulas containing hydrolysates having a degree of hydrolysis less than about 15% are commercially available from Nestlé Nutrition Company under the trade mark Peptamen®. Hydrolysates having a degree of hydrolysis
35 above about 15% may be prepared using the procedure described in EP 0322589.

5 The dietary protein hydrolysate source may also be in the form of a mix of free amino acids; preferably such that the mix provides a balanced amino acid profile. Free amino acids are preferably in an amount of about 0 to 20 % by weight of the total protein content (calculated as Nitrogen x 6.25).

Dietary protein in the form of a mix of free amino acids is found to increase the relative weight of the jejunum and the rate of protein synthesis in the jejunum.

10 The source of total proteins preferably provides about 5% to about 30% of the energy of the nutritional composition; for example about 10% to about 20% of the energy. The remaining energy of the nutritional composition may be provided in the form of carbohydrates and fats.

15 If the nutritional composition includes a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional composition; for example about 20% to about 50% of the energy. The lipids making up the fat source may be any suitable fat or fat mixture. Vegetable fats are particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins, and the like. Animal fats such as milk fats may also
20 be added if desired. The lipids may also include medium-chain triglycerides; for example up to about 60 % by weight of lipids as medium-chain triglycerides. Fractionated coconut oil is a suitable source of medium-chain triglycerides.

25 A source of carbohydrate may be added to the nutritional composition. It preferably provides about 40% to about 80% of the energy of the nutritional composition. Any suitable carbohydrates may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, and maltodextrins, and mixtures thereof.

30 Dietary fibre may also be added if desired. If used, it preferably comprises up to about 5% of the energy of the nutritional composition. The dietary fibre may be from any suitable origin, including for example soy, pea, oat, pectin, guar gum, and gum arabic.

35 Suitable vitamins and minerals may be included in the nutritional composition in an amount to meet the appropriate guidelines.

One or more food grade emulsifiers may be incorporated into the nutritional composition if desired; for example diacetyl tartaric acid esters of mono-diglycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included.

The nutritional composition is preferably enterally administrable; for example in the form of a powder, a liquid concentrate, a ready-to-drink, or a ready-to-administer beverage.

The nutritional composition may be prepared in any suitable manner. For example, it may be prepared by blending together the source of dietary protein, the carbohydrate source, and the fat source in appropriate proportions. If used, the emulsifiers may be included in the blend. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any lipophilic vitamins, emulsifiers and the like may be dissolved into the fat source prior to blending. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The temperature of the water is conveniently about 50°C to about 80°C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. The liquid mixture is then homogenised; for example in two stages.

The liquid mixture may then be thermally treated to reduce bacterial loads, by rapidly heating the liquid mixture to a temperature in the range of about 80°C to about 150°C for about 5 seconds to about 5 minutes, for example. This may be carried out by steam injection, autoclave or by heat exchanger; for example a plate heat exchanger.

Then, the liquid mixture may be cooled to about 60°C to about 85°C; for example by flash cooling. The liquid mixture may then be again homogenised; for example in two stages at about 7 MPa to about 40 MPa in the first stage and about 2 MPa to about 14 MPa in the second stage. The homogenised mixture may then be further cooled to add any heat sensitive components; such as vitamins and minerals. The pH and solids content of the homogenised mixture is conveniently standardised at this point.

If it is desired to produce a powdered nutritional composition, the homogenised mixture is transferred to a suitable drying apparatus such as a spray

drier or freeze drier and converted to powder. The powder should have a moisture content of less than about 5% by weight.

5 If it is desired to produce a liquid composition, the homogenised mixture is preferably aseptically filled into suitable containers by pre-heating the homogenised mixture (for example to about 75 to 85°C) and then injecting steam into the homogenised mixture to raise the temperature to about 140 to 160°C; for example at about 150°C. The homogenised mixture may then be cooled, for example by flash cooling, to a temperature of about 75 to 85°C. The
10 homogenised mixture may then be homogenised, further cooled to about room temperature and filled into containers. Suitable apparatus for carrying out aseptic filling of this nature is commercially available. The liquid composition may be in the form of a ready to feed composition having a solids content of about 10 to about 14% by weight or may be in the form of a concentrate; usually of solids
15 content of about 20 to about 26% by weight. Flavours may be added to the liquid compositions so that the compositions are provided in the form of convenient, flavoursome, ready-to-drink beverages.

20 In another aspect, this invention provides a method for increasing protein concentration and synthesis in the small intestine, the method comprising administering to a pre-mature or non-mature mammal an effective amount of a nutritional composition containing a dietary protein hydrolysates having a degree of hydrolysis of less than 50 % and intact proteins being partly in the form of bioactive peptides. Further, the dietary protein hydrolysate preferably has a non-
25 protein nitrogen concentration of at least about 85% of total nitrogen. Non protein nitrogen is defined as the nitrogen fraction not recovered as a precipitate after acidification.

30 Preferably, the method may be used to treat premature or non-mature young mammals to promote growth and maturation of the gastro-intestinal tract. Additionally, the method can also apply to situations encountered in clinical nutrition when alterations of the normal growth or turnover of the gut mucosa occur, e.g. after long term total parenteral nutrition or malnutrition.

35 The nutritional enteral composition also intends to cover very high nutrient needs for growth, development and maintenance during those situations. It

ensures optimal digestion and utilization (for tissue accretion) of the protein source and intends to minimize the nitrogen waste of the organism. The composition may also be used for patients with gut mucosa damage.

5 The amount of the nutritional composition to be administered will vary depending upon the state of maturation or growth of the gut of the mammal.

Example 1

Whole protein

10 An amount of 5 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in demineralised water at 55°C to obtain protein concentration (N*6.38) of 10% by weight. The pH of the dispersion is adjusted by the addition of 190 g of calcium hydroxide and the dispersion is cooled to room temperature. The proteins are then dried by lyophilisation and
15 packaged into metal cans.

The whole proteins have a degree of hydrolysis of about 4.41% and a non protein nitrogen concentration of about 1.1% on the basis of total nitrogen.

Hydrolysate 1

20 An amount of 6.25 kg of whey protein (obtained from Meggle GmbH) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 8.2 by the addition of 1.8 litres of 2M Ca(OH)₂. The proteins are then hydrolysed using 30 g of trypsin (Salt free pancreatic trypsin which has an activity of 6.8 AU/g and a chymotrypsin content of less than 5% and which is
25 obtainable from Novo Nordisk Ferment AG, Dittigen, Switzerland). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to
30 16°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans. The hydrolysate has a degree of hydrolysis of about 14% and a non protein nitrogen concentration of about 54.5% on the basis of total nitrogen.

Hydrolysate 2

35 An amount of 6.25 kg of whey protein (obtained from Meggle GmbH) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is

adjusted to 7.5 by the addition of 1.6 litres of 1M $\text{Ca}(\text{OH})_2$ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 50 g of trypsin (obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated and non-hydrolysed protein is denatured, by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes.

The mixture is then cooled to 56°C and hydrolysed again for 1 hour using 50g of trypsin at 55°C. During the reaction, the pH is regulated to 7.4 by the addition of 1.6N NaOH and 0.4N KOH. The enzymes are then inactivated by heating the reaction mixture to 80°C and holding the mixture at this temperature for about 5 minutes. The mixture is then cooled to 18°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

The hydrolysate has a degree of hydrolysis of about 17.3% and a non protein nitrogen concentration of about 65.9% on the basis of total nitrogen.

Hydrolysate 3

An amount of 6.25 kg of whey protein (obtained from Meggle GmbH under the trade name Globulal 80) is dispersed in 50 litres of demineralised water at 55°C. The pH of the dispersion is adjusted to 7.5 by the addition of 1.6 litres of 1M $\text{Ca}(\text{OH})_2$ and 162 ml of a solution of 1.6M NaOH and 0.4M KOH. The proteins are then hydrolysed using 250 g of Alcalase 2.4L (EC 940459 - obtainable from Novo Nordisk Ferment AG). The hydrolysis reaction is continued for 4 hours at 55°C. For the first hour of the reaction, the pH is regulated to 7.6 by the addition of 1.6N NaOH and 0.4N KOH.

An amount of 250g of Neutrase 0.5L (obtainable from Novo Nordisk Ferment AG) is added and the proteins are further hydrolysed for 4 hours at 50°C. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5 minutes. The reaction mixture is then cooled to 55°C.

The pH of the reaction mixture is adjusted to 7.33 by the addition of 1.6N NaOH and 0.4N KOH and the reaction mixture hydrolysed again for 4 hours using 100g of pancreatin at 55°C. During the reaction, the pH is regulated to 7.5 by the addition of 1M NaOH. The enzymes are then inactivated by heating the reaction mixture to 90°C and holding the mixture at this temperature for about 5

minutes. The mixture is then cooled to 4°C. The hydrolysed proteins are then dried by lyophilisation and packaged into metal cans.

5 The hydrolysate has a degree of hydrolysis of about 35% and a non protein nitrogen concentration of about 92.6% on the basis of total nitrogen.

Example 2

10 In order to obtain a nutritional composition intended for specific gastro intestinal maturation in premature mammals, the following mixture is prepared :

i) 14.5 g/ 100 g powder total protein content:

- 10 10 % hydrolysate 2 as prepared in example 1,
- 40 % hydrolysate 3 as prepared in example 1,
- 15 50 % intact proteins (containing 1 ppm TGFβ2),

ii) 26 g/ 100 g powder of fat:

- 40 % medium chain triglycerides
- 20 60 % long chain triglycerides

iii) 53.6 g/ 100 g powder carbohydrates

- 65 % lactose
- 35 % maltodextrins

25 iv) and vitamins, minerals to meet daily requirements.

Claims

1. A nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals, which contains as a protein source a mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides.
2. A composition according to claim 1, wherein the dietary protein hydrolysates are in the form of a mixture of different size peptides, free amino acids or a mixture thereof.
3. A composition according to claim 2, wherein the dietary protein hydrolysates contain at least about 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least about 5 % of hydrolysates having a lesser degree of hydrolysis.
4. A composition according to claims 2 or 3, wherein free amino acids are in an amount of about 0 to about 20 % by weight of the total protein content (calculated as Nitrogen x 6.25).
5. A composition according to any of claims 1 to 4, wherein the intact proteins are in an amount of at least about 5% by weight of the total protein content (calculated as Nx6.25).
6. A composition according to any of claims 1 to 5, wherein the intact proteins are milk proteins, whey proteins, caseins and bioactive peptides such as TGF- β 2.
7. A composition according to any of claims 1 to 6, wherein bioactive peptides represent at least about 0.1 to about 4 ng/mg total protein.
8. A composition according to any of claims 1 to 7 which contains a source of protein providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.

9. Use of a selected mixture of dietary protein hydrolysates and intact proteins being partly in the form of bioactive peptides as protein source in the preparation of a nutritional enteral composition intended for favoring the growth and maturation of non-mature gastro-intestinal tracts of young mammals.
10. Use according to claim 9, wherein the dietary protein hydrolysates are in the form of a mixture of different size peptides, free amino acids or a mixture thereof.
11. Use according to claim 9 or 10, wherein the dietary protein hydrolysates comprise at least 5 % (by weight, of the total protein content calculated as Nitrogen x 6.25) of hydrolysate having a degree of hydrolysis of about 40 and at least 5 % of hydrolysates having a lesser degree of hydrolysis.
12. Use according to any of claim 9 to 11, wherein free amino acids are in an amount of about 0 to about 20 % by weight of the total protein content (N x 6.25)
13. Use according to any of claims 9 to 12, wherein the intact proteins are in an amount of at least about 5% of the total protein content.
14. Use according to any of claims 9 to 13, wherein the the intact proteins are milk proteins, whey proteins, caseins and bioactive peptides such as TGF- β 2.
15. Use according to any of claims 9 to 14, wherein bioactive peptides represent about 0.1 to about 4 ng/mg total protein.
16. Use according to any of claims 9 to 15, in which the nutritional composition contains a source of protein providing 5 to 30% of the total energy, a source of carbohydrates which provides 40 to 80% of the total energy, a source of lipids which provides 5 to 55% of the total energy, minerals and vitamins to meet daily requirements.

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PTO/PCT Rec'd 12 SEP 2001

BECKER KURIG STRAUS
BAVARIASTRASSE 7 - 80336 MÜNCHEN

18. Juni 2001

WV: / LF:

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**NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**
(PCT Rule 71.1)

Date of mailing:
(day/month/year) 15.06.2001

Applicant's or agent's file reference

NO 6436/WO 80300 WO

IMPORTANT NOTIFICATION

International application No.

PCT/EP00/01744

International filing date (day/month/year)

01/03/2000

Priority date (day/month/year)

12/03/1999

Applicant

SOCIETE DES PRODUITS NESTLE S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



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Authorized officer

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NO 6436/WO	<div style="display: flex; justify-content: space-between;"> <div> FOR FURTHER ACTION </div> <div> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) </div> </div>	
International application No. PCT/EP00/01744	International filing date (day/month/year) 01/03/2000	Priority date (day/month/year) 12/03/1999
International Patent Classification (IPC) or national classification and IPC A23J3/34		
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 4 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 2 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application 		
Date of submission of the demand 06/10/2000	Date of completion of this report 15.06.2001	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 </div> </div>	Authorized officer De Jong, E Telephone No. +31 70 340 3849	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International

ation No. PCT/EP00/01744

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-10 as originally filed

Claims, No.:

1-7 as received on 05/03/2001 with letter of 05/03/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International

cation No. PCT/EP00/01744

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-7
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations

see separate sheet

Ad I

The subject-matter of the claims does not meet the requirements of Rule 70.2(c) PCT, because there is no support in the original disclosure for the phrase "degree of hydrolysis in a range of from about 10% to less than 50% by weight" (underlining added) in claim 1.

Ad V

Reference is made to the following documents:

D1 = US-A-4 977 137

D2 = US-A-5 514 655

D3 = CA-A-2 163 379

D4 = Hamosh M., Journal of Nutrition 1997, p.971S-974S

D5 = Schanbacher F.L. et al., International Dairy Journal 1998, p.393-403

D6 = EP-A-0 852 913

The subject-matter of the claims is considered to be anticipated and not to involve an inventive step (Articles 33(2) and (3) PCT) in view of D1-D6:

D1 discloses (see claims 1-27) the use of lactoferrin as a dietary ingredient to a formula, e.g. hydrolysed casein formula (see col.11 l.54-col.12 l.23), to promote growth of the gastrointestinal tract of human infants and newborn nonhuman animals immediately on birth.

D2 discloses (see claims 1-2, col.19 l.1-67) a nutritional enteral product which contains a protein system comprising a soy protein hydrolysate and intact protein (sodium caseinate, pea protein, whey protein concentrate). The product provides enteral nutritional support for cancer patients.

D3 discloses a nutritional composition for oral or enteral administration, comprising a source of dietary nitrogen providing 15-25% of the total energy, carbohydrates providing 60-75% of the total energy and lipids providing 10-20%. The dietary nitrogen component comprises 20-30% by weight free amino acids, 60-75% hydrolysed casein and 5-15% intact caseinate protein. The composition provides improved digestion and adsorption.

D4-D6 refer to the importance of bioactive peptides for infant formulas and functional foods (e.g. casein rich in TGF-[SPEC0803]2 for treatment of Crohn's disease).

PCT

PTO/PCT Rec'd 12 SEP 2001 INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference NO 6436/WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/01744	International filing date (day/month/year) 01/03/2000	(Earliest) Priority Date (day/month/year) 12/03/1999
Applicant SOCIETE DES PRODUITS NESTLE S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23J3/34 A23J3/305

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23J A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, FSTA, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 977 137 A (NICHOLS BUFORD L ET AL) 11 December 1990 (1990-12-11) column 11, line 54 -column 12, line 23; claims 1-27	1-16
X	US 5 514 655 A (SNOWDEN GREGORY A ET AL) 7 May 1996 (1996-05-07)	1-8
Y	column 19, line 1-67; claims 1,2	9-16
X	CA 2 163 379 A (SANDOZ NUTRITION LTD ;SCHMIDL MARY KATHRINE (US); HAHN DOUGLAS E () 24 May 1996 (1996-05-24)	1-8
Y	page 4-5	9-16
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
 "&" document member of the same patent family

Date of the actual completion of the international search

29 June 2000

Date of mailing of the international search report

12/07/2000

Name and mailing address of the ISA

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Authorized officer

De Jong, E

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HAMOSH M: "Should infant formulas be supplemented with bioactive components and conditionally essential nutrients present in milk?" JOURNAL OF NUTRITION, vol. 127, no. 5S, Suppl., 1997, pages 971S-974S, XP002112500 Dep. of Pediatrics, Georgetown Univ. Med. Cent., Washington, DC 20007, USA table 1</p>	9-16
Y	<p>SCHANBACHER F L ET AL: "Milk-borne bioactive peptides." 4691, USA, XP002112501 /6) 393-403 1998 Dep. of Animal Sci., Lab. of Molecular & Dev. Biol., Ohio Agric. Res. & Dev. Cent., Ohio State Univ., Wooster, OH 44691, USA the whole document</p>	9-16
Y	<p>EP 0 852 913 A (NESTLE SA) 15 July 1998 (1998-07-15) claims 1-10</p>	9-16
A	<p>EP 0 322 589 A (NESTLE SA) 5 July 1989 (1989-07-05) cited in the application claims 1-12</p>	1-16
A	<p>EP 0 631 731 A (SQUIBB BRISTOL MYERS CO) 4 January 1995 (1995-01-04) claims 1-22</p>	1-16
A	<p>EP 0 827 697 A (NESTLE SA) 11 March 1998 (1998-03-11) claims 1-8</p>	1-3

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 4977137	A	11-12-1990	AU 618265 B	19-12-1991
			AU 1735388 A	08-12-1988
			CA 1315675 A	06-04-1993
			DE 3888023 D	07-04-1994
			DE 3888023 T	22-09-1994
			EP 0295009 A	14-12-1988
			JP 1093534 A	12-04-1989
US 5514655	A	07-05-1996	AT 179609 T	15-05-1999
			AU 650821 A	30-06-1994
			CA 2105788 A,C	29-11-1994
			DE 69324799 D	10-06-1999
			DE 69324799 T	02-12-1999
			EP 0626175 A	30-11-1994
			ES 2132158 T	16-08-1999
			GR 3030752 T	30-11-1999
			IL 106977 A	18-06-1996
			JP 6335364 A	06-12-1994
			JP 7121206 B	25-12-1995
			KR 9705331 B	15-04-1997
			NZ 248604 A	22-12-1994
			ZA 9306594 A	18-05-1994
CA 2163379	A	24-05-1996	NONE	
EP 0852913	A	15-07-1998	AU 5182698 A	16-07-1998
			CA 2223198 A	14-07-1998
			JP 10203996 A	04-08-1998
			US 5952295 A	14-09-1999
EP 0322589	A	05-07-1989	EP 0321603 A	28-06-1989
			AT 84680 T	15-02-1993
			AU 2659688 A	29-06-1989
			CA 1334064 A	24-01-1995
			DE 3877733 A	04-03-1993
			DE 3877733 T	09-06-1993
			ES 2053690 T	01-08-1994
			JP 2002319 A	08-01-1990
			JP 6077504 B	05-10-1994
			MX 169602 B	14-07-1993
			PH 26140 A	18-03-1992
			PT 89325 A,B	29-12-1989
			US 5039532 A	13-08-1991
			ZA 8809280 A	27-09-1989
EP 0631731	A	04-01-1995	US 5405637 A	11-04-1995
			AU 672697 B	10-10-1996
			AU 6598894 A	12-01-1995
			CA 2126639 A	31-12-1994
			CN 1105818 A,B	02-08-1995
			JP 7023717 A	27-01-1995
			US 5589357 A	31-12-1996
EP 0827697	A	11-03-1998	NONE	